

Listing of Claims.

Please amend the claims as shown below by deleting the material indicated by strike-through and adding the underlined material. This listing of claims will replace all prior versions and listings of the claims in this application.

1. (Original) A method for screening a candidate compound for its ability to interact with at least one transmembrane protein comprising:

transfecting a cell with at least one nucleotide sequence encoding a protein comprising a transmembrane protein containing at least one nuclear localisation sequence (NLS) and a detectable moiety and permitting expression of the encoded protein in the cell;

contacting the cell with a candidate compound; and

determining the distribution of the expressed protein in the cell by detecting the distribution of the detectable moiety in the cell;

wherein detection of an altered distribution of the detectable moiety in the cell relative to the distribution of the detectable moiety in a control cell not contacted with the candidate compound indicates that the compound interacts with the transmembrane protein.

2. (Previously presented) The method of claim 1 wherein the detectable moiety is a detectable peptide comprising an antigenic portion of the amino acid sequence of the transmembrane protein and/or wherein the nucleotide sequence encodes a fusion protein comprising a transmembrane protein containing at least one NLS and a detectable moiety.

3. (Cancelled)

4. (Previously presented) The method of claim 1 wherein the wild type transmembrane protein contains an NLS.

5. (Previously presented) The method of claim 1 wherein the wild type transmembrane protein lacks an NLS and the nucleotide sequence encoding the transmembrane protein is modified to encode an NLS.
6. (Currently amended) The method of claim 5 wherein the nucleotide sequence is modified to encode an NLS selected from Table 1 or wherein the nucleotide sequence is modified to encode an amino acid sequence selected from the group consisting of KKFKR (SEQ ID NO: 158 157), PKKKRKV (SEQ ID NO: 154 129) and AFSAKKFKR (SEQ ID NO: 159 158).
7. (Cancelled)
8. (Previously presented) The method of claim 1 wherein the cell is a prokaryotic cell or a eukaryotic cell selected from the group consisting of a mammalian cell, optionally selected from the group consisting of HEK, COS and CHO cells, a yeast cell, an insect cell, a nematode cell, a plant cell and a fungal cell.
- 9-10. (Cancelled)
11. (Previously presented) The method of claim 2 wherein the detectable moiety is an antigenic peptide and the distribution of the antigenic peptide in the cell is determined by allowing it to bind to an antibody-based detection system comprising an antibody specific for the antigenic peptide, optionally an antibody-based detection system comprising a first antibody specific for the antigenic peptide and a second antibody carrying a detectable label and specific for the first antibody or an antibody-based detection system comprising a first antibody specific for the antigenic peptide and carrying a detectable label, optionally an optically detectable label, optionally a luminescent or fluorescent label.

12-15. (Cancelled)

16. (Original) The method of claim 3 wherein the detectable moiety is a polypeptide selected from the group consisting of green fluorescent protein, red fluorescent protein and modified variants thereof.

17. (Previously presented) The method of claim 1 wherein the transmembrane protein is selected from the group consisting of a G protein coupled receptor (GPCR), a transporter, a cytokine receptor, a tyrosine kinase receptor and a low density lipoprotein (LDL) receptor.

18. (Original) The method of claim 17 wherein the transmembrane protein is a GPCR.

19. (Original) The method of claim 18 wherein the GPCR is selected from the group consisting of dopamine D1 receptor, dopamine D2 receptor, dopamine D3 receptor, dopamine D5 receptor, histamine 1 receptor, cysteinyl leukotriene receptor 1, cysteinyl leukotriene receptor 2, opioid receptor, muscarinic receptor, serotonin receptor, beta2-adrenergic receptor and metabotropic glutamate 4 receptor.

20. (Previously presented) The method of claim 17 wherein the transmembrane protein is a transporter selected from the group consisting of dopamine transporter and serotonin transporter, a cytokine receptor selected from the group consisting of erythropoietin receptor and insulin receptor, a tyrosine kinase receptor selected from the group consisting of epidermal growth factor receptor and insulin receptor or a low density lipoprotein receptor.

21-26. (Cancelled)

27. (Previously presented) The method of claim 1 wherein the cell is transfected with a plurality of nucleotide sequences, each of said sequences encoding a protein comprising a different transmembrane protein containing at least one NLS and wherein each of said nucleotide sequences encodes a protein comprising a different detectable moiety or wherein at least one detectable moiety is common to at least two encoded proteins.

28-29. (Cancelled)

30. (Original) The method of claim 1 wherein the cell is contacted with a compound known to interact with the at least one transmembrane protein prior to contacting the cell with the candidate compound and

wherein detection of an altered distribution of the detectable moiety in the cell relative to the distribution of the detectable moiety in a control cell contacted with the compound known to interact with the transmembrane protein but not contacted with the candidate compound indicates that the candidate compound interacts with the transmembrane protein.

31. (Previously presented) The method of claim 1 wherein detection of an altered distribution of the detectable moiety in the cell comprises detection of a reduced level or an increased level of the detectable moiety associated with the cell membrane.

32-33. (Cancelled)

34. (Previously presented) The method of claim 1 wherein detection of an altered distribution of the detectable moiety in the cell comprises detection of a

reduced level or an increased level of the detectable moiety in the nucleus of the cell.

35-36. (Cancelled)

37. (Original) A method for screening a candidate compound for its ability to interact with at least one transmembrane protein comprising:

transfecting a cell with at least one nucleotide sequence encoding an NLS-containing transmembrane protein and permitting expression of the encoded protein in the cell;

contacting the cell with a candidate compound; and

determining the level of NLS-containing transmembrane protein remaining at the cell membrane by isolating the cell membrane fraction of the cell, contacting the fraction with a labelled ligand of the transmembrane protein and determining the level of binding of the ligand to the fraction;

wherein detection of an altered level of the transmembrane protein at the cell membrane relative to the level at the cell membrane in a control cell not contacted with the candidate compound indicates that the compound interacts with the transmembrane protein.

38. (Original) The method of claim 37 wherein the labelled ligand is a radio-labelled ligand.

39. (Previously presented) The method of claim 37 wherein the wild type transmembrane protein contains an NLS.

40. (Previously presented) The method of claim 37 wherein the wild type transmembrane protein lacks an NLS and the nucleotide sequence encoding the transmembrane protein is modified to encode an NLS.

41. (Previously presented) The method of claim 40 wherein the nucleotide sequence is modified to encode an NLS selected from Table 1 or wherein the nucleotide sequence is modified to encode an amino acid sequence selected from the group consisting of KKFKR, PKKKRKV and AFSAKKFKR.

42. (Cancelled)

43. (Previously presented) The method of claim 37 wherein the cell is a prokaryotic cell or a eukaryotic cell selected from the group consisting of a mammalian cell, optionally selected from the group consisting of HEK, COS and CHO cells, a yeast cell, an insect cell, a nematode cell, a plant cell and a fungal cell.

44-45. (Cancelled)

46. (Previously presented) The method of claim 37 wherein the transmembrane protein is selected from the group consisting of a G protein coupled receptor (GPCR), a transporter, a cytokine receptor, a tyrosine kinase receptor and a low density lipoprotein (LDL) receptor.

47. (Original) The method of claim 46 wherein the transmembrane protein is a GPCR.

48. (Original) The method of claim 47 wherein the GPCR is selected from the group consisting of dopamine D1 receptor, dopamine D2 receptor, dopamine D3 receptor, dopamine D5 receptor, histamine 1 receptor, cysteinyl leukotriene receptor 1, cysteinyl leukotriene receptor 2, opioid receptor, muscarinic receptor, serotonin receptor, beta2-adrenergic receptor, and metabotropic glutamate 4 receptor.

In re: O'Dowd et al.
Application No.: 10/509,787
Filed: May 23, 2005
Attorney Docket No. 3477-110
Page 9 of 19

49. (Previously presented) The method of claim 46 wherein the transmembrane protein is a transporter selected from the group consisting of dopamine transporter and serotonin transporter, a cytokine receptor selected from the group consisting of erythropoietin receptor and insulin receptor, a tyrosine kinase receptor selected from the group consisting of epidermal growth factor receptor and insulin receptor or a low density lipoprotein receptor.

50-55. (Cancelled)

56. (Previously presented) The method of claim 37 wherein the cell is transfected with a plurality of nucleotide sequences, each of said sequences encoding a protein comprising a different transmembrane protein containing at least one NLS and wherein each of said nucleotide sequences encodes a protein comprising a different detectable moiety or wherein at least one detectable moiety is common to at least two encoded proteins.

57-58. (Cancelled)

59. (Previously presented) The method of claim 37 wherein detection of an altered distribution of the detectable moiety comprises detection of a reduced level or an increased level of the detectable moiety associated with the cell membrane.

60. (Cancelled)

61. (Original) An isolated cell transfected with at least one nucleotide sequence encoding a protein comprising a transmembrane protein containing at least one NLS and a detectable moiety.

In re: O'Dowd et al.
Application No.: 10/509,787
Filed: May 23, 2005
Attorney Docket No. 3477-110
Page 10 of 19

62. (Previously presented) The cell of claim 61 wherein the detectable moiety is a detectable peptide comprising an antigenic portion of the amino acid sequence of the transmembrane protein and/or wherein the nucleotide sequence encodes a fusion protein comprising a transmembrane protein containing at least one NLS and a detectable moiety.

63. (Cancelled)

64. (Previously presented) The cell of claim 61 wherein the wild type transmembrane protein contains an NLS.

65. (Previously presented) The cell of claim 61 wherein the wild type transmembrane protein lacks an NLS and the nucleotide sequence encoding the transmembrane protein is modified to encode an NLS.

66. (Previously presented) The cell of claim 65 wherein the nucleotide sequence is modified to encode an NLS selected from Table 1 or wherein the nucleotide sequence is modified to encode an amino acid sequence selected from the group consisting of KKFKR, PKKKRKV and AFSAKKFKR.

67. (Cancelled)

68. (Previously presented) The cell of claim 62 wherein the detectable moiety is an antigenic peptide and the distribution of the antigenic peptide in the cell is determined by allowing it to bind to an antibody-based detection system comprising an antibody specific for the antigenic peptide, optionally an antibody-based detection system comprising a first antibody specific for the antigenic peptide and a second antibody carrying a detectable label and specific for the first antibody or an antibody-based detection system comprising a first antibody specific for the antigenic peptide

and carrying a detectable label, optionally an optically detectable label, optionally a luminescent or fluorescent label.

69-72. (Cancelled)

73. (Original) The cell of claim 63 wherein the detectable moiety is a polypeptide selected from the group consisting of green fluorescent protein, red fluorescent protein and modified variants thereof.

74. (Previously presented) The cell of claim 61 wherein the transmembrane protein is selected from the group consisting of a G protein coupled receptor (GPCR), a transporter, a cytokine receptor, a tyrosine kinase receptor and a low density lipoprotein (LDL) receptor.

75. (Original) The cell of claim 74 wherein the transmembrane protein is a GPCR.

76. (Original) The cell of claim 75 wherein the GPCR is selected from the group consisting of dopamine D1 receptor, dopamine D2 receptor, dopamine D3 receptor, dopamine D5 receptor, histamine 1 receptor, cysteinyl leukotriene receptor 1, cysteinyl leukotriene receptor 2, opioid receptor, muscarinic receptor, serotonin receptor, beta2-adrenergic receptor and metabotropic glutamate 4 receptor.

77. (Previously presented) The cell of claim 74 wherein the transmembrane protein is a transporter selected from the group consisting of dopamine transporter and serotonin transporter, a cytokine receptor selected from the group consisting of erythropoietin receptor and insulin receptor, a tyrosine kinase receptor selected from the group consisting of epidermal growth factor receptor and insulin receptor or a low density lipoprotein receptor.

78-83. (Cancelled)

84. (Previously presented) The cell of claim 61 transfected with a plurality of nucleotide sequences, each of said sequences encoding a protein comprising a different transmembrane protein containing at least one NLS and wherein each of said nucleotide sequences encodes a protein comprising a different detectable moiety or wherein at least one detectable moiety is common to at least two encoded proteins.

85-86. (Cancelled)

87. (Previously presented) The cell of claim 61 wherein the cell is a prokaryotic cell or a eukaryotic cell selected from the group consisting of a mammalian cell, optionally selected from the group consisting of HEK, COS and CHO cells, a yeast cell, an insect cell, a nematode cell, a plant cell and a fungal cell.

88-89. (Cancelled)

90. (Previously presented) The cell of claim 87 wherein the cell is a neuronal cell.

91. (Previously presented) A compound identified as capable of interacting with a transmembrane protein by the method of claim 1.

92. (Original) A method for determining whether a first protein and a second protein are able to oligomerise comprising:

transfected a cell with a first nucleotide sequence encoding a first protein containing an NLS and a second nucleotide sequence encoding a second protein

In re: O'Dowd et al.
Application No.: 10/509,787
Filed: May 23, 2005
Attorney Docket No. 3477-110
Page 13 of 19

comprising a detectable moiety and permitting expression of the encoded first and second proteins in the cell; and

determining the distribution of the detectable moiety in the cell;
wherein detection of the detectable moiety in or adjacent to the nucleus of the cell or detection of a reduced level of the detectable moiety at the cell surface, relative to a control cell, indicates that the first and second proteins interact.

93. (Previously presented) The method of claim 92 wherein the first and second proteins are different transmembrane proteins or are the same transmembrane protein or wherein one of the first and second proteins is a transmembrane protein and the other is a non-transmembrane protein.

94-95. (Cancelled)

96. (Previously presented) The method of claim 92 wherein the first and second proteins are GPCRs.

97. (Previously presented) The method of claim 92 wherein the detectable moiety is a detectable peptide comprising an antigenic portion of the amino acid sequence of the second protein or wherein the second nucleotide sequence encodes a fusion protein comprising the second protein and a detectable moiety.

98. (Cancelled)

99. (Previously presented) The method of claim 92 wherein the wild type first protein contains an NLS.

In re: O'Dowd et al.
Application No.: 10/509,787
Filed: May 23, 2005
Attorney Docket No. 3477-110
Page 14 of 19

100. (Previously presented) The method of claim 92 wherein the wild type first protein lacks an NLS and the first nucleotide sequence encoding the first protein is modified to encode an NLS.

101. (Previously presented) The method of claim 100 wherein the first nucleotide sequence is modified to encode an NLS selected from Table 1 or wherein the first nucleotide sequence is modified to encode an amino acid sequence selected from the group consisting of KKFKR, PKKKRKV and AFSAKKFKR.

102. (Cancelled)

103. (Previously presented) The method of claim 92 wherein the cell is a prokaryotic cell or a eukaryotic cell selected from the group consisting of a mammalian cell, optionally selected from the group consisting of HEK, COS and CHO cells, a yeast cell, an insect cell, a nematode cell, a plant cell and a fungal cell

104-105. (Cancelled)

106. (Previously presented) The method of claim 92 wherein the detectable moiety is an antigenic peptide and the distribution of the antigenic peptide in the cell is determined by allowing it to bind to an antibody-based detection system comprising an antibody specific for the antigenic peptide, optionally an antibody-based detection system comprising a first antibody specific for the antigenic peptide and a second antibody carrying a detectable label and specific for the first antibody or an antibody-based detection system comprising a first antibody specific for the antigenic peptide and carrying a detectable label, optionally an optically detectable label, optionally a luminescent or fluorescent label.

107-111. (Cancelled)

112. (Original) The method of claim 92 wherein the detectable moiety is a polypeptide selected from the group consisting of green fluorescent protein, red fluorescent protein and modified variants thereof.

113. (Previously presented) The method of claim 92 wherein the first and second proteins are transmembrane proteins selected from the group consisting of a G protein coupled receptor (GPCR), a transporter, a cytokine receptor, a tyrosine kinase receptor and a low density lipoprotein (LDL) receptor.

114. (Previously presented) The method of claim 113 wherein at least one transmembrane protein is a GPCR selected from the group consisting of dopamine D1 receptor, dopamine D2 receptor, dopamine D3 receptor, dopamine D5 receptor, histamine 1 receptor, cysteinyl leukotriene receptor 1, cysteinyl leukotriene receptor 2, opioid receptor, muscarinic receptor, serotonin receptor, beta2-adrenergic receptor, and metabotropic glutamate 4 receptor.

115. (Previously presented) The method of claim 113 wherein at least one transmembrane protein is a transporter selected from the group consisting of dopamine transporter and serotonin transporter, a cytokine receptor selected from the group consisting of erythropoietin receptor and insulin receptor, a tyrosine kinase receptor selected from the group consisting of epidermal growth factor receptor and insulin receptor or a low density lipoprotein receptor.

116-121. (Cancelled)

122. (Original) The method of claim 92 wherein the first nucleotide sequence encodes a first protein further comprising a detectable moiety different from the detectable moiety of the second protein, wherein detection of an energy transfer

In re: O'Dowd et al.
Application No.: 10/509,787
Filed: May 23, 2005
Attorney Docket No. 3477-110
Page 16 of 19

interaction between the detectable moiety of the first protein and the detectable moiety of the second protein indicates that the first and second proteins oligomerise.